

Using otolith shape analysis to distinguish barracudas *Sphyraena sphyraena* and *Sphyraena viridensis* from the Algerian coast

by

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Abstract. – Otolith shape analyses were conducted on two species of barracudas (*Sphyraena sphyraena* and *Sphyraena viridensis*) from the Gulf of Annaba (south-western Mediterranean). The otolith shape was described by elliptic Fourier descriptors from 14 harmonics and by five indices of shape (coefficient of form, roundness, circularity, rectangularity and ellipticity). The comparison through canonical discriminant analyses (CDA) was performed between species and between right and left otoliths. The CDA demonstrated strong discrimination of the two species, with significant differences and a high classification success. The percentage of well-classified individuals in predefined groups was higher than 80%. The combined use of morphometric variables (from indices) and external outlines (shape analysis through Fourier series) demonstrated the importance of otolith shape for interspecific discrimination.

Résumé. – Différenciation de deux espèces de barracudas (*Sphyraena sphyraena* et *Sphyraena viridensis*) des côtes de l'Est algérien par l'analyse de la forme des otolithes.

La forme des otolithes de deux espèces de barracudas (*Sphyraena sphyraena* et *Sphyraena viridensis*) du golfe d'Annaba (Algérie Nord-Est) est décrite par 14 harmoniques à l'aide des descripteurs elliptiques de Fourier et par cinq indices de forme (coefficient de forme, rondeur, circularité, rectangularité et ellipticité). L'analyse canonique discriminante est appliquée sur les otolithes droit et gauche de tous les individus considérés. Elle discrimine significativement les deux espèces avec un pourcentage d'individus bien classés supérieur à 80% dans les groupes prédéfinis. L'utilisation combinée de variables morphométriques (taille et forme) et des contours externes (analyse de forme à l'aide des séries de Fourier) montre l'importance de la forme des otolithes dans la discrimination interspécifique.

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In the Mediterranean Sea, the *Sphyraena* genus includes five species: *S. sphyraena* (Linnaeus, 1758), *S. chrysotaenia* Klunzinger, 1884, *S. flavicauda* Rüppell, 1838 and *S. viridensis* Cuvier, 1829 (Tortonèse, 1979; Ben-Tuvia, 1986; Fisher *et al.*, 1987; Fredj and Maurin, 1987; Quignard and Tomasini, 2000; Golani *et al.*, 2002). *Sphyraena sphyraena* is present in the eastern Atlantic from the Bay of Biscay to Angola, and in the western Atlantic from Bermuda to Brazil (Fisher *et al.*, 1987). *S. chrysotaenia* is an Indo-Pacific species, considered a Lessepsian migrant, and is encountered only in the eastern Mediterranean basin (Golani, 1996). *Sphyraena flavicauda* was recorded by Golani (1992) in Israel, by Bilecenoglu *et al.* (2002) in Turkey (Antalya Bay) and later in Libya (Ben Abdallah *et al.*, 2005). *S. viridensis* is known to be present off the Eastern Central Atlantic: Cape Verde, Canary Islands and also in Azores Islands (Barreiros *et al.*, 2002). It is reported in the eastern Mediterra-

nean, from coasts of Lebanon, Egypt and Israel (George *et al.*, 1971; Tortonèse, 1979; Ben-Tuvia, 1986; Allam *et al.*, 2004). However, its exact distribution and abundance are unknown because most published records do not separate it from *Sphyraena sphyraena*. Pastore (2009) described a new species, *Sphyraena intermedia*, in the Gulf of Taranto in the central Mediterranean Sea. The latter is distinguished from its congeners by the form of its body, its otoliths, its teeth and its pyloric caeca (Pastore, 2009).

Recently, several studies have reported a broader distribution of *Sphyraena viridensis*, in the northern Mediterranean Sea (Bizsel and Cihangir, 1996; Relini and Orsi-Relini, 1997; Dulčić and Soldo, 2004; Kožul *et al.*, 2005; Psomadakis *et al.*, 2006; Dulčić *et al.*, 2009; Kalogirou *et al.*, 2012) and in the southern Mediterranean Sea (Corsini and Economidis, 1999; Vacchi *et al.*, 1999; Kara and Bourehail, 2003). The presence of the species in the southern Mediter-

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reanean Sea remained dubious for a long time (Fisher *et al.*, 1987), and both species, *S. viridensis* and *S. sphyraena*, were regarded in the literature as the juvenile and adult forms of the same species *S. sphyraena* (Bini, 1969; Tortonese, 1975; Bauchot and Pras, 1980). To eliminate this confusion and to verify the presence of these species along the Algerian coast, we attempted to discriminate them using otolith shape analyses.

Otoliths are concretions of calcium carbonate (CaCO_3) that are metabolically inert and unable to undergo either dissolution or resorption (Campana, 1999). They are located in the inner ear of teleosts, where they take part in mechano-reception and equilibration mechanisms (Popper and Coombs, 1980). Thus, the otoliths constitute the environment perception elements in fish. In addition, they record the life history features of the individuals (age, chemical elements, reproduction, etc.) and have been described as a “flight recorder” of fish (Lecomte-Finiger, 1999). Otoliths have been used in biology in many studies (Campana, 2005) for many species determinations (L’Abée-Lund, 1988), age estimation (Campana, 2001), growth (Lombarte and Morales-Nin, 1995), stock identification (Friendland and Reddin, 1994; Tracey *et al.*, 2006; Gonzalez-Salas and Lenfant, 2007), and diet assessment (Barrett *et al.*, 1990; Martucci *et al.*, 1993; Velando and Freire, 1999; Morat *et al.*, 2011).

Otolith shape is species specific (L’Abée-Lund, 1988; Campana and Casselman, 1993), and thus partially subject to genetics (Vignon and Morat, 2010). The study of otoliths allows species recognition, classification and identification (L’Abée-Lund, 1988; Lo-Yat, 2002; Campana, 2004; Lombarte *et al.*, 2006). Moreover, recent studies indicate that the otolith shape has a close relationship with environmental conditions (Hoff and Fuiman, 1993; Lombarte and Leonart, 1993; Cardinale *et al.*, 2004). They have also been used to characterise various local populations (Hoff and Fuiman, 1993; Lombarte and Leonart, 1993; Cardinal *et al.*, 2004; Morat *et al.*, 2012). In addition, the ecomorphologic link hypothesis of otolith shape was put forth in connection with the history features of fish life and the biological and behavioural characteristics of species, such as the type of habitat (Volpedo and Echeverria, 2003) or the type of swimming activity (Lychakov and Rebane, 2000; Lo-Yat, 2002). Otolith shape applications are not limited to ichthyology, but are widely extended to the study of the feeding ecology of fish predators, and to some aspects of palaeontology, stratigraphy, archaeology and zoogeography (Schwarzahns, 1999). Koken (1884) described in detail and pictured the otoliths of 32 recent species, belonging to 26 genera, as well as otoliths from a considerable number of fossil species. In our study, first, the otoliths of *Sphyraena* species collected along the Algerian coast were described, and second, the otolith

shapes of *S. sphyraena* and *S. viridensis* were used for clarifying the presence of these species in this area.

MATERIALS AND METHODS

Studied areas and sampling

A total of 91 *S. sphyraena* (176–425 mm L_T , mean $L_T = 297 \text{ mm} \pm 32.8 \text{ mm}$) and 103 *S. viridensis* (254–888 mm L_T , mean $L_T = 422 \text{ mm} \pm 129 \text{ mm}$) were sampled in the Gulf of Annaba in the southwestern Mediterranean Sea ($36^\circ 54' \text{N}$, $7^\circ 45' \text{E}$) (Fig. 1) between 2001 and 2005. The right and left sagittae of all individuals (338 otoliths in total) were extracted using fine forceps, washed with distilled water and stored dry in referenced tubes. The total length (L_T in mm) of the fish was measured.

Shape analysis

Each otolith was examined under a stereomicroscope (Leica Wild M8) fitted with a digital camera (XC-77CE) linked to a computer. An episcopic light through optical fibres allowed optimization of the direction and intensity of light to obtain the most highly contrasted image. Digital images were then acquired with the software, Visilog (v. 5.6, Noésis), which also calculated the surface area of the otolith (A_o), its perimeter (P_o), its length (maximum measure, L_o) and its width (maximum measure, l_o) to the nearest 10^{-2} mm . These measures allowed the calculation of five shape indices

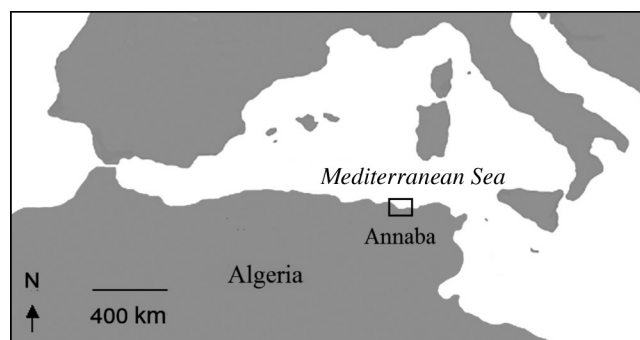


Figure 1. - Location of studied site in the South-Western Mediterranean Sea.

Table I. - Shape indices calculated in this study (from Tuset *et al.*, 2003b). A_o : area of the otolith; L_o : length; l_o : width; P_o : perimeter.

Shape indices	Formulae
Coefficient of form	$4\pi A_o / P_o^2$
Roundness	$(4A_o) / (\pi L_o^2)$
Circularity	P_o^2 / A_o
Rectangularity	$A_o / (L_o \times l_o)$
Ellipticity	$(L_o - l_o) / (L_o + l_o)$

(Tab. I), which are independent from differences in otolith size (see hereafter) (Russ, 1990; Tuset *et al.*, 2003a, b).

The shape of each otolith was assessed with the elliptic Fourier analysis (Stransky and MacLellan, 2005). This technique describes the outline on the basis of several components named harmonics. Each harmonic is characterised by four coefficients, resulting from the projection of each point of the outline on axes (x) and (y). The higher the number of harmonics, the greater the accuracy of the outline description is (Kuhl and Giardina, 1982). For each digital image, the software Shape 1.2 (Iwata and Ukai, 2002) calculated the Fourier coefficients to make them invariants to the otolith size and its orientation (and position) regarding the beginning of the outline, which is arbitrarily defined. In addition, the Fourier power (FP) spectrum was calculated to determine the sufficient and necessary number of harmonics for the best reconstruction of the otolith outline (Crampton, 1995). The Fourier power of a harmonic is proportional to its amplitude and provides a measure of the amount of “shape information” described by this harmonic. For the n th harmonic, the Fourier power (FP $_n$) is given by the expression:

$$FP_n = (A_n^2 + B_n^2 + C_n^2 + D_n^2) / 2$$

where A_n , B_n , C_n and D_n are the Fourier coefficients of the n th harmonic. Then, we can calculate the cumulated power percentage (FP $_c$) as follows:

$$FP_c = \sum_1^n FP_n$$

For this purpose, two sub-samples of 30 otoliths each (the first for right otoliths and the second for left otoliths) were randomly chosen for the two species, and the threshold of 99.99% of the mean cumulated Fourier power was chosen to define the suitable number of harmonics to be considered in the analyses (Crampton, 1995). As the first 14 harmonics reached 99.99% of the cumulated power for both the right and left otoliths, the Fourier analysis indicated that the otolith shape of the studied species could be summarised by these 14 harmonics, i.e., 56 Fourier coefficients. However, the coefficients derived from the first harmonic were not taken into account because the outline reconstructed with these coefficients is a simple ellipse resulting in maximum Fourier power, which then masks the information derived from the other harmonics (Crampton, 1995). Therefore, 13 harmonics and 52 Fourier coefficients were used for the data analyses.

Data analysis

The analytical design was set up to determine differences between species through canonical discriminant analysis. The CDA was performed with the 52 Fourier coefficients. The objective of CDA is to investigate the integrity of predefined groups, i.e., individuals belonging to a given group such as species, through finding linear combinations of descriptors that maximise the Wilks lambda (λ) (Ramsay and Silveman, 2005). The Wilks λ allows assessment of the performance of

the discriminant analysis. This statistic is the ratio between the intra-group variance and the total variance and provides a means of calculating the chance-corrected percentage of agreement between true and predicted group membership. The values of λ range from 0 to 1, and the closer the λ is to 0, the better the discriminating power of the CDA.

Several CDAs were performed, first to search for the best subset of data to increase the quality of the discrimination process. CDAs were performed with data from the right otoliths, with data from the left otoliths, and with data from both otoliths pooled. The first step was to discriminate the two species. The second step was to determine the relative positions of the right and the left otolith of each species. Euclidian distances (chosen as they allow graphic representation of the observations according to the first discriminant scores from a discriminant analysis) between the barycentre of different groups were calculated to quantify the dissimilarity between groups, and the reclassifying rate of individuals within the predefined groups was determined with a Cohen's kappa test that expresses the average proportion of individuals with index agreement, after removing the effect of chance agreement (Titus *et al.*, 1984).

RESULTS

Otolith morphology

For the two species of barracudas studied here, the sagittae were lanceolated and thin (Fig. 2). The inner face was convex, and the outer face was flat. The dorsal margin was sinuate (rounded with regular wave-like curves) with the highest point at about its middle and formed a rounded right angle with the posterior margin. The posterior margin was rounded ventrally and may have a flattened dorsal part in larger otoliths. The ventral margin was rounded. The *sulcus* (longitudinal groove on proximal surface) was divided into an anterior *ostium* and a posterior *cauda* by constriction of the dorsal and ventral margins of the *sulcus*. The *sulcus* was *ostial* (opens onto the anterior-dorsal margin), and the *cauda* had a poorly defined tip. The *ostium* and *cauda* were distinct, and the *ostium* was bulbous ventrally and larger than the *cauda*, a condition known as heterosulcoid. The *cauda* flared dorsally towards its tip. The crista superior (dorsal rim of *sulcus*) was well developed to ridge-like over the *ostium* and anterior *cauda*. The crista inferior (ventral rim) was well developed to ridge-like under the *cauda*. The rostrum was broad and rounded and the antirostrum small. They were separated by a narrow, sharp *excisura*.

Shape indices

The mean distances of descriptors tested separately in both species demonstrated a significant difference between the right and left otoliths of *S. sphyraena*, highlighted by the

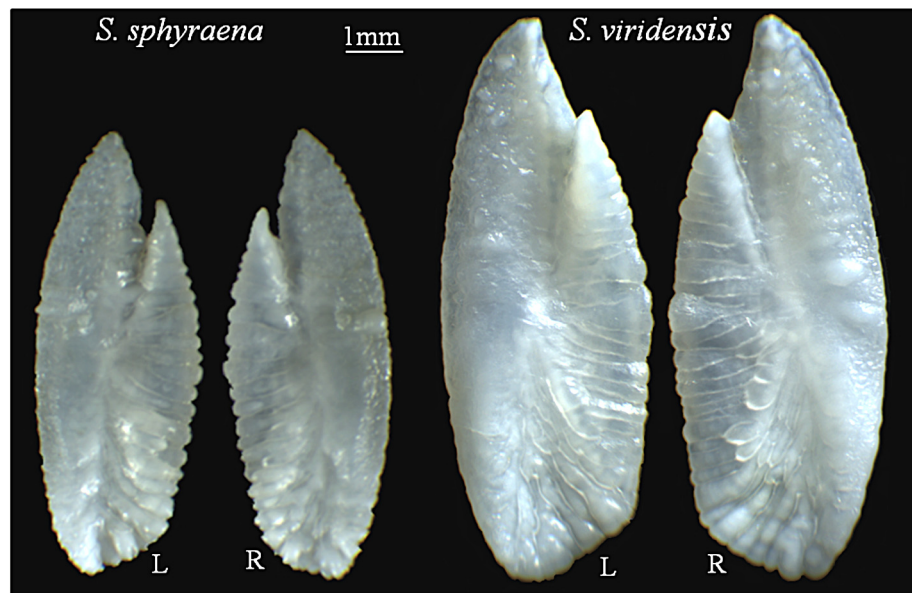


Figure 2. - Photographs of left (L) and right (R) sagittal otoliths of *S. sphyraena* ($L_T = 37$ cm) and *S. viridensis* ($L_T = 53$ cm), captured in the Gulf of Annaba.

Table II. - Comparisons of the mean values ($\pm$ SD) of the five shape indices between left and right otoliths for the two species, *S. sphyraena* ($n = 24$) and *S. viridensis* ($n = 27$), analysed by t-test.

Shape indices	Species	Mean $\pm$ SD		<i>t</i>	d. f.	<i>p</i>
		Left	Right			
Coefficient of form	<i>Sphyraena viridensis</i>	0.333 ± 0.018	0.338 ± 0.021	0.777	26	0.444
	<i>Sphyraena sphyraena</i>	0.381 ± 0.039	0.372 ± 0.035	-2.616	23	0.015
Roundness	<i>Sphyraena viridensis</i>	0.343 ± 0.026	0.342 ± 0.026	0.171	26	0.865
	<i>Sphyraena sphyraena</i>	0.332 ± 0.019	0.333 ± 0.017	-0.958	23	0.349
Circularity	<i>Sphyraena viridensis</i>	37.741 ± 2.091	37.281 ± 2.411	-0.783	26	0.441
	<i>Sphyraena sphyraena</i>	33.340 ± 3.586	34.010 ± 3.466	2.379	23	0.026
Rectangularity	<i>Sphyraena viridensis</i>	0.718 ± 0.015	0.722 ± 0.013	1.240	26	0.226
	<i>Sphyraena sphyraena</i>	0.727 ± 0.018	0.727 ± 0.022	-0.395	23	0.696
Ellipticity	<i>Sphyraena viridensis</i>	0.454 ± 0.034	0.457 ± 0.033	0.857	26	0.400
	<i>Sphyraena sphyraena</i>	0.471 ± 0.020	0.470 ± 0.020	0.473	23	0.641

Table III. - Summary of the results from the discriminant analysis performed between right and left otoliths of the two species.

	Fourier descriptors		
	F1	F2	F3
λ	0.061	0.308	0.647
P-value	< 0.001	< 0.001	< 0.001
Percentage of inertia	70.91%	19.47%	9.63%

shape indices and circularity. No significant dimorphism was observed in *S. viridensis* (Tab. II).

Species discrimination

With all samples, the first axis of the discriminant analysis represented 70.91% of the variability ($\lambda_1 = 0.061$, $p < 0.001$) (Tab. III). This allowed discrimination of the two species *S. sphyraena* and *S. viridensis* from the shape of their otoliths. The Cohen-Kappa test indicated a high rate

of reclassification among the origin groups ($85.8 \pm 5.3\%$). The left and right otoliths of the species *S. viridensis* were also differentiated by the second axis ($\lambda_2 = 0.308$, $p < 0.001$). On the contrary, the right and left otoliths of *S. sphyraena* species were not discriminated. The graphical representation of the discrimination obtained from all analysed descriptors of otoliths of both species is presented in figure 3. Three main groups can be distinguished. The two samples representing the left and right otoliths of the *S. sphyraena* species

were part of the same group, whereas those of *S. viridensis* were separated into two distinct groups. The primary function appeared to separate the two populations according to the shape of their otoliths.

DISCUSSION

To verify the presence of the two species *S. sphyraena* and *S. viridensis* along the Algerian coast, we discriminated them by using otolith shape analyses.

The sagittae of *S. sphyraena* and *S. viridensis* have a quite similar shape. However, some specific characteristics can be identified: (1) the dorsal edge of the otoliths of *S. viridensis* is more convex than that of *S. sphyraena*; (2) in *S. sphyraena*, the junction between the posterior edge and the dorsal edge is almost a right angle, but slightly more open

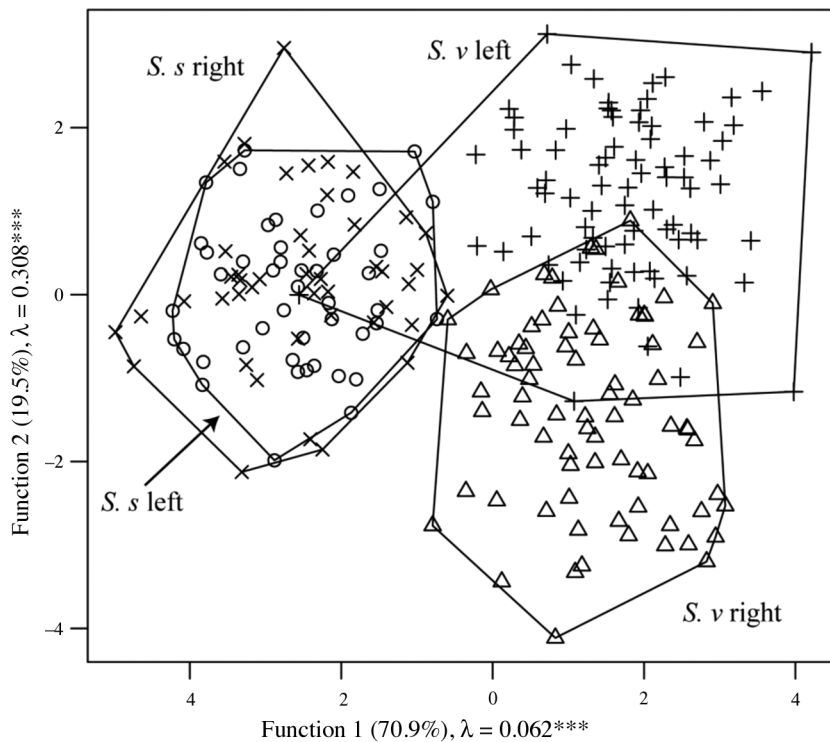


Figure 3. - Canonical discriminant analysis achieved with both otolith of the two species of Barracuda *Sphyræna sphyræna* (*S. s*) and *Sphyræna viridensis* (*S. v*) (x) *S. s* right, (o) *S. s* left, (+) *S. v* right and (Δ) *S. v* left.

than for *S. viridensis*, and likewise, the junction between the posterior edge and the ventral edge of the younger *sagittae* of *S. viridensis* can be a soft curve as in *S. sphyræna*; (3) the ventral edge is extremely convex in *S. sphyræna* and is slightly convex in *S. viridensis*.

The examination of the otoliths reveals a strong resemblance to the pair of *sagittae* 14-15 of *S. acutipinnis* Day, 1876 as illustrated by Rivaton and Bourret (1999: plate 67). The otolith morphology of the two species *S. sphyræna* and *S. viridensis* in the central Mediterranean Sea was described as similar by Pastore (2009). Nevertheless, a comparison with the *sagittae* of our sample and those described in the central eastern Atlantic and western Mediterranean by Tuset *et al.* (2008) makes it possible to highlight a number of differences between the two species: (1) for *S. sphyræna*, the junction between the posterior edge and the dorsal edge is almost at a right angle but slightly more open than for *S. viridensis*; (2) the *ostium* of the *sulcus* in both right and left otoliths is largely open for the two studied species (*S. sphyræna* and *S. viridensis*). Its form is funnel-like, shorter than the *cauda* for the two species of *Sphyræna* described by Tuset *et al.* (2008); and (3) the junction between the posterior edge and the ventral edge of the younger *sagittae* of *S. viridensis* can be a soft curve as with *S. sphyræna*. Indeed, the circularity makes it possible to differentiate both species according to their geographic origin. This variable varies from 33.3 to 37.7 for specimens of *S. sphyræna* and *S. viridensis* of the

Gulf of Annaba, respectively, and from 21.0 to 21.9 for the fish described by Tuset *et al.* (2008).

Asymmetry of otoliths

The otoliths of the investigated species (*S. sphyræna* and *S. viridensis*) did not display significant differences in weight between left and right *sagittae*, which is in contrast with the findings of Harvey *et al.* (2000) for *Lycodes palearis*. Even the weight difference between right and left otoliths is small, which may not amplify the symmetric function of the otolith system. Therefore, we do not deny that functional asymmetries exist. Asymmetry could be due to other differences in hair cell sensitivity, in neural connection and in the efferent system (Takabayashi and Ohmura-Iwasaki, 2003). The important point of the otolith asymmetry hypothesis is that the structural and/or physiological asymmetries are compensated for on earth, and this compensation is lost in microgravity (Takabayashi and Ohmura-Iwasaki, 2003).

Growth-related differences in linear otolith proportions have been described for other species (Reznick *et al.*, 1989; Secor and Dean, 1989). These differences could have many origins; Schwarzhans (1994) referred to the sexual dimorphism in otoliths of ophidiid genus (*Neobythites*). The faster growing males of this genus have more elongate and thinner otoliths. Gauldie and Nelson (1990) described differences in otolith shape as a function of parasite load for the orange roughy (*Hoplostethus atlanticus* Collett, 1889). Slow-growing parasitized fish had thicker and wider otoliths. Wilson

(1985), describing compensatory depth-related differences in otolith sizes for macrourids from the Atlantic and Pacific oceans, found that otoliths were either long and thin, or short, wide, and thick. The shape differences noted by Wilson may be attributable to regional and depth-related differences in temperature and/or fish growth rates. In our case, the difference of growth rate between both species studied (unpublished data) can partially explain the different types of allometry.

There were significantly different mean circularity values between the two species; the value is 33 in *S. sphyraena* and 37 in *S. viridensis*. Tuset *et al.* (2003a) found similar results, except for roundness in three species of the genus *Serranus*. They explained this pattern by the depth range of the species. Small and therefore young individuals were characterised by a more jagged and a spindle-shaped otolith outline than the adults, which conversely displayed a more or less oval outline (Mérigot *et al.*, 2007).

The coefficient of form displayed an overlap between the two species *S. sphyraena* and *S. viridensis* (0.37 and 0.33, respectively), highlighting the variability of otolith outlines within each species. This could be due to the heterogeneity of fish sizes within both species samples. Several authors have provided evidence of the role of fish size in explaining variability in otolith outline (Castanguay *et al.*, 1991; Campana and Casselman, 1993; Begg *et al.*, 2001; Cardinale *et al.*, 2004).

Discrimination of *S. sphyraena* and *S. viridensis*

In this study, the discriminant analysis indicated a clear differentiation in otolith shape between the two species of Barracuda *S. sphyraena* and *S. viridensis* with more than 80% of individuals correctly classified. Different results are common in the literature. Friedland and Reddin (1994) consider only discriminant values of > 75% as acceptable. The 75% accuracy for *Cynoscion nebulosus* (Cuvier, 1830) were reported for the Galveston Bay system using scale and otolith shape Fourier descriptors (Colura and King, 1995). Simoneau *et al.* (2000) obtained values of up to 70% accuracy analysing differentiation of six populations of lake trout *Salvelinus namaycush* in Canadian rivers, whereas a greater classification success of 56–81% was reported in year classes in the identification of haddock *Melanogrammus aeglefinus* stocks from George Bank (Begg and Brown, 2000). De Vries *et al.* (2002) obtained 81.6% accuracy in differentiating stocks of king mackerel *Scomberomorus cavalla* between the eastern Gulf of Mexico and Atlantic Ocean. Tuset *et al.* (2003a) demonstrated that regional differences in the comber *Serranus cabrilla* existed between the Canary Islands and Alicante with an accuracy of 68.8%. The estimate of Cohen's kappa indicated that these success rates were significantly better than the 50% expected by chance alone.

In conclusion, in this study, the data indicated a high discrimination rate. This suggests that variability in otolith shape is a good tool for species identification in *S. sphyraena* and *S. viridensis* because the mean value of the classification percentage (80%) is comparable to those obtained in other studies using the same type of analysis (Friedland and Reddin, 1994; Colura and King, 1995; De Vries *et al.*, 2002; Tuset *et al.*, 2003a, b). Thus, further research is needed, including genetic studies to complement information collected on these species.

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